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(54) Title: ANTIBODIES TO NON-FUNCTIONAL P2X$_7$RECEPTOR, DIAGNOSIS AND TREATMENT OF CANCERS AND OTHER CONDITIONS

(57) Abstract: The invention concerns a wide range of diseases and conditions, including cancers. The invention provides a probe for detection of such a disease or condition. The probe is able to distinguish between functional P2X$_7$ receptors and non-functional P2X$_7$ receptors. The probe can do this in various ways, one of which is detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors. The invention also provides a method for detecting the disease or condition, using the probe. The invention extends to treatment of the disease or condition, using an antibody, or an epitope capable of generating the antibody, which can distinguish between functional and non-functional P2X$_7$ receptors and bind to the non-functional receptors. Methods of treatment, pharmaceutical compositions and use of the probe and antibody are also included.

Antibodies to non-functional $P2X_7$ receptor, diagnosis and treatment of cancers and other conditions

TECHNICAL FIELD

This invention concerns diagnosis and treatment of diseases, including cancers. The types of diseases with which this invention is concerned include cancers derived from epithelial cells and malignant lymphoma. The invention also concerns other conditions, such as preneoplastic states, irritable bowel syndrome and viral and other infections. It is quite possible that the invention is also applicable to other diseases and conditions.

BACKGROUND

Adenosine triphosphate (ATP) can activate ligand-gated purinergic receptors known as P2X receptors. Receptor subtypes $P2X_1$ to $P2X_7$ have been identified. It is known that different P2X receptor subtypes are present in many cells, including epithelial cells and leukocytes, including lymphocytes, thymocytes, macrophages and dendritic cells.

P2X receptors are permeable to calcium ions as well as some other cations, such as potassium and sodium. An influx of calcium ions into a cell via a P2X receptor can be associated with cell death.

It is believed that the $P2X_7$ subtype is involved in apoptosis, or programmed cell death, in many cell types. In the presence of ATP, the $P2X_7$ receptor expressed on the surface of a cell is capable, within a second, of opening calcium channels through the cell membrane. Continued exposure to ATP can lead to the formation of large pores, within a few seconds to tens of seconds, that enable the cell to be flooded with excess calcium, inducing apoptosis.

The amino acid sequences of the human and rat $P2X_7$ receptors are known, for example, from US patent No. 6,133,434 (Buell et al). Refer also to Figure 1 herein.

Exposure to ATP does not generally result in apoptosis in the case of epithelial cancer cells, for example. It has been found that such cells express $P2X_7$ receptors that are unable to form pores. These are regarded as non-functional receptors.

In human cancer cell lines, such as prostate PC3 and breast MCF7, as well as in animal cell lines including rodent hybridomas, the $P2X_7$ receptor is found on the cell surface in a non-functional conformation.

The B-cells of patients with malignant lymphoma express non-functional $P2X_7$ receptors. Lymphoma develops from malignant clones that escape cytolytic destruction. This process leads to the progressive accumulation of malignant B-lymphocytes and thus lymphadenopathy and/or splenomegaly.

SUMMARY OF THE INVENTION

In a first aspect, this invention provides a probe for detection of a disease or condition, the probe being adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors. Preferably, the probe distinguishes between functional and non-functional $P2X_7$ receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors or by detecting change in binding of one or more proteins necessary for pore formation in $P2X_7$ receptors. In an alternate embodiment, the probe detects one or more parts of the $P2X_7$ receptor exposed in the absence of bound ATP. Such receptor part may include a $P2X_7$ monomer.

The invention also provides a method for detecting a disease or condition, the method including the steps of using the probe of the invention to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors, providing a receptor expression profile, and comparing the receptor expression profile with that of a normal profile. The change may be detected, for example, as indicated above in connection with the probe itself.

The probe may be natural or artificial. Preferably, the probe is an antibody, which may be polyclonal, monoclonal, recombinant, a humanised antibody, a human antibody or an appropriate fragment thereof. The antibody is preferably directed against an epitope located in an extracellular domain adjacent to a site for binding ATP. In the case of human $P2X_7$ receptors, the probe is preferably adapted to distinguish between functional receptors having a sequence in which proline at amino acid 210 is in the trans conformation and non-functional receptors having a sequence in which the proline at amino acid 210 is in the cis conformation that acts to impart a significant alteration in the local protein structure.

The probe may be prepared using any suitable technique, as will be readily apparent to one skilled in the art.

It is within the scope of the invention that the probe may distinguish between functional and non-functional receptors through detection of other conformational changes occurring at a site for binding ATP. For example, the change detected may be in an amino acid other than the proline referred to above. An example of such an amino acid is Pro199 which, when in the cis conformation, significantly alters the local protein structure. As another example, the change detected may be in some other respect.

The probe may also be adapted to detect other regions of the $P2X_7$ receptor unchanged by functional state. The conformation of the monomeric subunits lacking bound ATP may be detectable using the probe, as the epitope chosen may specifically detect the shape of a region of the surface of the receptor accessible only when ATP is not bound. The probe may detect change in binding of one or more proteins, such as accessory or other proteins, necessary for pore formation. Non-limiting examples of such proteins are laminin, integrin, beta-actin, alpha-actinin and supervillin.

In the present invention, a $P2X_7$ subtype-specific antibody can be used to specifically detect or bind to non-functional $P2X_7$ receptors expressed in or on cells

forming part of preneoplastic tissue, very early neoplastic tissue, advanced neoplastic tissue and on any neoplastic cell expressing non-functional $P2X_7$ receptors. Thus, the $P2X_7$ receptor is detected or bound only when in the close-gated or non-functional conformation, even though it may be normally expressed in the cell membranes and may otherwise be partially able to function as a channel.

Further, the conformation of the monomeric subunits lacking bound ATP is also detectable with the antibody, because the epitope chosen specifically detects the shape of a region of the surface accessible only when ATP is not bound.

In the present invention, the non-functional $P2X_7$ receptors can be detected or bound by using an antibody directed against an epitope that undergoes a conformational change from the structure present in functional receptors. It has been found that the amino acid sequence of the non-functional receptors can be identical to the amino acid sequence of functional receptors, so that the cause of the conformational change in the receptors relates to the interaction of the receptors with ATP. As set out above, the ATP molecules act as receptor agonists, so that when ATP is bound to the receptors, they are able to open a channel through the cell membrane for the inflow of calcium ions. Non-functionality is therefore caused by a lack of appropriate binding of the ATP agonists to the receptors, for reasons that may include a deficit in the local availability of ATP through production deficit or increase in the rate of degradation. If ATP binding to the receptors is disrupted, the receptor conformation is altered. This can be detected by using an antibody specially designed to bind to the region of the protein affected by the binding of the ATP.

In the case of human $P2X_7$ receptors, the specific sequence involved in the conformational change may include Pro210, which undergoes a change in conformation from the trans form to the cis form in the absence of bound ATP. Thus, in the case of human receptors, an appropriate epitope sequence against which an antibody must be raised may include Pro210, and may extend either side

of this residue, to an appropriate extent necessary to induce an antibody response. By way of non-limiting example, this may include a segment extending from Gly200 to Cys216. Further, a homologous segment from other mammals, such as rat, may be used where this cross-reacts with human tissue. As an example, the same segment Gly200 to Cys216 in rat may be used, although there are two amino acid substitutions in the rat sequence compared with the human sequence (refer US patent No. 6,133,434, for example).

In the case of non-human receptors, the specific sequence may be ascertained by suitable experiment.

The detection of non-functional $P2X_7$ receptors according to the invention may show a distribution pattern in which functional receptors (and hence normal cells) may remain essentially unlabelled. However, non-functional conformations of $P2X_7$ receptors may be detected, initially in the nuclei and cytoplasm of cells, at a very early stage in preneoplasia. For example, in the case of epithelial cell cancer, using the method of the invention it may be possible to detect preneoplasia several years prior to the normal pathological appearance of cancer as detected by haematoxylin and eosin ("H & E") stained slides of biopsied tissues. Thus, cancers such as prostate, skin and breast may be detected far earlier than is currently the case, with the advantages of introduction of early therapy.

The full scope of the diseases and conditions which may be detected by the probe and method of the invention has not yet been ascertained. However, it is believed that these include epithelial cell cancers, such as prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, as well as blood cancers including malignant lymphoma, irritable bowel syndrome and infection by viruses such as HIV or other pathological organisms, such as Mycobacterium tuberculosis. Infection may cause non-functional receptors to be expressed either directly through inhibition of co-factors required for functionality, or through the

up-regulation of co-factors acting to inhibit $P2X_7$ function on epithelial or other cells, so rendering the infected cell less amenable to destruction by apoptosis.

Unless otherwise indicated, the term "disease or condition" as used herein is intended to include all those specific diseases and conditions set out in the preceding paragraph.

In the specific case of irritable bowel syndromes ("IBS"), it has now been found that, in patients with this condition, the gut mucosa, that normally expresses $P2X_7$ receptors in the widely distributed lymphocytes present in the stroma beneath the epithelium, becomes up-regulated. In affected patients, this increased expression can be observed from duodenum to rectal mucosa. The increased expression may be found in isolated regions, or to be generally increased over the entire length of the intestinal tract in more extreme cases.

In the least affected cases, total $P2X_7$ receptors are up-regulated, but these are all functional and they do not penetrate into the epithelium. In more severe cases, total $P2X_7$ receptor expression is even higher, and the most affected areas of the gut exhibit receptors that are non-functional. These may be localised to caecal mucosa, for example, and may penetrate into the epithelium. The most severe cases are those in which total $P2X_7$ receptor expression is further increased and most of the receptors are non-functional with increased epithelial cell penetration.

As already discussed, non-functionality of $P2X_7$ receptors is caused by lack of appropriate binding of the ATP agonist to the receptors. The reasons for this may include a deficit in the local availability of ATP through production deficit or increase in rate of degradation through ecto-ATPase enzymatic degradation of ATP. If ATP binding to the receptors is disrupted, the receptor conformation is altered as already discussed, and this can be detected using the probe of the invention. However, the detection of total $P2X_7$ receptor distribution is best achieved using an epitope to other regions of the extracellular domain of the $P2X_7$ receptor that is not affected by ATP binding. The probe may be capable of

detecting regions of the $P2X_7$ receptor unchanged by functional state, by detecting an epitope common to both functional and non-functional conformations, such as Val65-Lys81.

It is within the scope of this invention to use one or two $P2X_7$ subtype-specific antibodies to specifically distinguish between total $P2X_7$ distribution and the proportion of receptors that are non-functional and expressed in gut mucosa. Thus the two antibodies used together can detect both total receptor count and those receptor channels present only in a close-gated or non-functional conformation. The first antibody is adapted to detect total $P2X_7$ receptor expression. The probe comprising or attached to the antibody of the invention can provide the second antibody for detection of IBS, not only distinguishing between functional and non-functional $P2X_7$ receptors, but also allowing for detection of other regions in which the receptor is unchanged by functional state. The antibodies may be used separately or together. Preferably, they are used in combination.

The detection of all $P2X_7$ receptors, separately from non-functional $P2X_7$ receptors, determines the severity of the condition. Expression of non-functional $P2X_7$ receptors in the gastrointestinal mucosa occurs in a pattern in which normal cells remain essentially unlabelled. Thereafter, the non-functional conformation of $P2X_7$ is first detected in the stroma underneath the epithelium ranging from isolated patches in mild cases of the syndrome to extensive expression throughout the length of the gastrointestinal tract with isolated patches of infiltration of non-functional receptors into the epithelium.

The invention also provides a method of diagnosing irritable bowel syndrome, comprising detecting the $P2X_7$ expression profile of cells and/or tissue and comparing the profile with a predetermined expression profile of normal cells and/or tissue. Preferably, the detection of the $P2X_7$ expression profile includes use of one or more antibodies. Further, it is preferred that such antibody or antibodies are different from the probe of the invention in that they do not detect change in

relation to binding of ATP to the $P2X_7$ receptors. The preparation of such antibodies will be readily apparent to one skilled in the art.

The invention also includes use of one or more antibodies to diagnose irritable bowel syndrome.

Therapeutic treatment for this condition is discussed below, in connection with the third aspect of this invention.

The diagnostic can be used in standard microscopy employing standard immunohistochemical techniques. The diagnostic may also be used *in vivo*.

Diagnosis using the probe and method of the invention may be carried out using *in situ* imaging techniques to detect distribution in body tissues. In addition, standard microscopy, confocal microscopy and fluorescence activated cell sorting may be used. Normal immunohistochemical techniques for testing lymph, prostate, breast, skin, lung, uterus, bladder, cervix, stomach, oesophagus and similar biopsies, also fine needle aspirates of breast and other tissue and cell smears such as those taken for the detection of cervical cancer, may be used.

For *in vivo* diagnosis, it is preferred that the probe is a human antibody or domain, manufactured with no animal components. The antibody is preferably labelled with a short-lifetime radiolabel, detectable by means of scanning technology such as positron emission tomography (PET scanner). Such imaging can detect the aggregation of labelled antibody anywhere in the body, thus signalling the presence of non-functional receptors, associated with the presence of any tumour. Ideally, such a test should be conducted only after detection of primary cancer and for the purpose of checking for secondary cancer, or after a general screen by means of a blood test (refer below) has detected the likelihood of the presence of one of more tumours.

The probe and method of the invention may be employed to provide a blood test for detecting non-functional $P2X_7$ receptors and hence cancer or pre-cancerous

conditions. By way of example, the probe in the form of a fluorescent labelled antibody (monoclonal or polyclonal) can be used in flow cytometry against blood cell fractions of the patient in order to detect binding to non-functional receptors on various gated leukocytes, including T lymphocytes, B lymphocytes or macrophages.

In another form of blood test, the probe preferably takes the form of a labelled antibody attached to a matrix provided in a kit, enabling detection by the presence of a colour reaction to the binding of the fixed antibody to positive white blood cells. Such a kit may be suitable for use by medical practitioners.

In a similar blood test, the antibody probe of the invention may be used as a diagnostic tool for screening patients who may not have cancer but in whom the normal cell killing pathways are inhibited through lack of function in $P2X_7$ on one or more leukocytes. Such patients may express non-functional receptors on macrophages, indicating inhibition of the ability of those macrophages to kill infected cells, such as those infected by organisms like Mycobacterium tuberculosis, or other infectious agents including malaria and HIV. Such organisms preferentially proliferate in patients for whom the normal cell killing pathways are inhibited through lack of function in $P2X_7$ on one or more leukocytes.

Other techniques may be used with the probe and method of the invention.

This invention provides an antibody for treating a disease or condition, the antibody being adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors and being adapted to bind only to non-functional receptors. Preferably, the antibody distinguishes between the functional and non-functional receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors, or by detecting change in binding of one or more proteins necessary for pore formation in $P2X_7$ receptors and being adapted to bind only to non-functional receptors. In another embodiment, the antibody

distinguishes between the functional and non-functional receptors by detecting parts of the receptor exposed in the absence of bound ATP.

The antibody for treating diseases and conditions may be the same as the antibody which may be used as the probe for diagnosing diseases and conditions. Such an antibody could be used to treat skin cancers topically, for example. For systemic treatment of cancer, the antibody or its active fragments should be human or a human domain, in order to minimise undesirable immune response side effects.

The antibody of the invention may be used to treat diseases or conditions in mammals, including humans. Examples of the diseases or conditions have been set out above in connection with the probe of the invention.

The invention also provides an epitope capable of causing the generation of the antibody of the second aspect of the invention. The epitope preferably includes Pro210 and encompasses the segment Gly200 to Cys216 (in the $P2X_7$ sequence of the human receptor). The epitope should preferably have attached to the C-terminal end a Cys residue (Cys216) that is cross-linked to diphtheria toxin via the chemical cross-linker maleimidocaproyl-N-hydroxysuccinimide (MCS), so that the conformation adopted by the attached epitope peptide occupies a stable cis proline configuration.

This specific peptide conformation is intended to be presented to humans or animals with one or more diseases or conditions, especially epithelial cell cancers, such as prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, as well as malignant lymphoma, irritable bowel syndrome and infection by viruses such as HIV or other pathological organisms, such as Mycobacterium tuberculosis. The patient will preferably mount an immune response to the applied conjugated epitope and so generate antibodies recognising the non-functional $P2X_7$ receptors present on the surface of the affected cells, thus binding to them and alerting the appropriate immune cell to destroy the complexed cells. Other cells primed for cell death may also be affected.

It is to be understood that the sequence referred to above is not limiting on the scope of the invention, which includes alternate sequences and carriers and cross-linkers that similarly produce a specific immune response, preferably against only non-functional $P2X_7$ receptors, preferably ignoring all functional receptors expressed on cell surfaces, and so avoiding side effects.

The invention, in this second aspect, also provides for the use of the antibody of the invention as a therapeutic vehicle for treatment of a disease or condition in a patient to regulate programmed cell death by targeting aberrant or non-functional $P2X_7$ receptors expressed on the surface of cells, while leaving all cells expressing normal (functional) receptors untouched. The invention also covers the use of the epitope of the invention to cause the generation of the antibody, as above.

The invention also provides a pharmaceutical composition for treatment or prevention of a disease or condition in a patient, the composition including a pharmaceutically effective amount of an antibody, or an epitope to cause the generation of such an amount, capable of regulating programmed cell death of cells having expressed on their surface aberrant or non-functional $P2X_7$ receptors.

The pharmaceutically effective amount of the antibody or epitope will vary according to the patient and the nature of the disease or condition. These variables can be ascertained by one skilled in the art.

The pharmaceutical composition of the invention may be administered in conjunction with a pharmaceutically acceptable carrier, which may be any of those known in the art or devised hereafter and suitable for the intended use. As well as carriers, the pharmaceutical compositions of the invention may include other ingredients, including dyes, preservatives, buffers and antioxidants, for example.

The pharmaceutical composition of the invention may take any desired form and may be administered, for example, in the form of an ointment, cream, solution, suspension, powder, tablet, capsule, suppository or pessary.

The pharmaceutical composition of the invention may be administered in any suitable way, which may include oral, parenteral, intravenous, intramuscular, subcutaneous or topical administration.

The invention also provides a method of treating or preventing a disease or condition in a patient, the method including administering to the patient a pharmaceutical composition according to the invention.

The invention also provides the use of the pharmaceutical composition of the invention, in the treatment or prevention of a disease or condition, in a patient.

It will be apparent to one skilled in the art that the pattern of use of the pharmaceutical composition of the invention may need to be altered for optimum effect. It may be necessary to take into account the nature of the disease or condition as well as its severity.

The third aspect of the invention focuses on the expression of ATPases (enzymes) that control the supply of ATP to $P2X_7$ receptors, for example in the B-cells of a patient having malignant lymphoma. Channel opening of $P2X_7$ receptors on leukocytes is terminated through the rapid hydrolysis of ATP agonist by ecto-ATPases and ecto-ATPdiphosphohydrolases (ecto-ATPDases). These enzymes regulate numerous physiological processes that are dependent on ATP. Substrate specificity of ATPase and ATPDase activity on lymphocytes indicates the presence on the lymphocytes of more than one type on the cell surface, including CD39. Proliferation of one or more of these ATPases or ATPDases could limit the supply of ATP needed to control $P2X_7$ pore formation and the subsequent programmed cell death needed to regulate B-cell numbers.

Similarly, it is believed that, in the case of IBS, proliferation of ATPases may contribute to lack of appropriate binding of the agonist ATP to the $P2X_7$ receptors.

Accordingly, in this third aspect, the invention provides a preparation for treatment or prevention of a disease or condition in a patient, the preparation including one or

more substances adapted to regulate the expression of ATPases that control the supply of ATP to $P2X_7$ receptors in the patient's cells or tissues. The invention also provides a method of treating or preventing a disease or condition in a patient, the method including the step of administering to the patient a preparation including one or more substances adapted to regulate the expression of ATPases that control the supply of ATP to $P2X_7$ receptors in the cells or tissue of the patient.

Examples of such ATPases may be CD39 or CD73.

Such a substance may take the form of an ATP analogue, preferably non-hydrolysable, and specific for $P2X_7$, or another substance that inhibits the action of local ATPases depleting the availability of ATP for the $P2X_7$ binding site. The preparation may be in the form of a human antibody directed specifically against non-functional $P2X_7$ receptors.

A substance such as an ATP analogue may bind to the $P2X_7$ and hold it in open pore configuration, thus forcing the pore to assume a functional state, in which it is able to take up both large and small cation permeants. In this way the use of such a synthetic agonist may act to restore receptor function, at the same time as controlling the growth advantage that $P2X_7$ provides cells in its role as a calcium channel.

The disease or condition is preferably malignant lymphoma or IBS but the invention may also extend to other diseases or conditions, including other epithelial cell or blood cancers or viral and other pathological infections.

In the case of malignant lymphoma, the ATPases control the local supply of ATP to the $P2X_7$ receptors so as to reduce the concentration of ATP available for binding to the $P2X_7$ receptors and so deactivate them leading to a significant reduction in programmed B-cell death. These ATPases may be specifically expressed on the surface of the B-cells and appear to be up-regulated in malignant lymphoma. Preferably, application of a specific ATPase inhibitor may be used to

regulate the availability of ATP on the $P2X_7$ receptors, so regulating programmed B-cell death.

For treatment of malignant lymphoma, the substance may include a synthetic agonist capable of blocking ATPases or ATPDases, of the form of non-hydrolysable $P2X_7$ agonist.

In relation to irritable bowel syndrome, administration of the preparation of the invention is intended to restore receptor function that may be depleted through overactivity of the muscle underlying the affected region of mucosa. The preparation of the invention may act on the mucosa directly to remove these non-functional receptors and thereby restore local normal gastrointestinal secretory mechanisms. Therapeutic treatment is aimed at restoring the local supply of ATP to the non-functional receptors, so that normal receptor function is restored. The consequences of control of receptor function include restoration of normal control of gastrointestinal secretions and peristalsis. This may be achieved by application of enteral or systemic supply of synthetic $P2X_7$-specific agonist, preferably non-hydrolysable by ATPases, by systemic application of an antibody directed against non-functional $P2X_7$ receptors, preferably a small human specific antibody to remove the non-functional receptors, leaving only functional receptors.

If abnormalities of peristalsis in the underlying smooth muscle are responsible for depleting the local availability of ATP for binding to the normal $P2X_7$ receptors, treatment may involve restoration of this natural supply of agonist by means of a limit on the uptake or use of ATP by the smooth muscle through application of a treatment to temporarily limit gut motility.

The invention also provides a pharmaceutical composition for treatment of a disease or condition, the composition including a pharmaceutically effective amount of one or more substances adapted to regulate the expression of ATPases (enzymes) that control the supply of ATP to $P2X_7$ receptors.

The invention in all its aspects extends to such similar applications that could be made in other medical conditions in which aberrant $P2X_7$ receptors are involved as a result of viral infection where the virus is protected in the infected cell by up-regulating non-functional $P2X_7$ receptor or where such receptors are up-regulated from the normal cell condition.

The invention also provides a method of treating irritable bowel syndrome, comprising administering to a patient a pharmaceutical composition as defined above.

The invention also provides the use of such a pharmaceutical composition in the treatment of irritable bowel syndrome.

The pattern of use of one or more of the above pharmaceutically effective agents may need to be altered for optimum effect.

Expressed another way, the invention provides a method of treating irritable bowel syndrome, the method including administering a composition adapted to restore $P2X_7$ receptor function. The receptor function may have been depleted through overactivity of the muscle underlying the affected region of mucosa. The composition may be the same as that set out above for the substance included in the preparation of the invention.

In a further aspect, the invention provides a method for distinguishing between different conformations of proteins by using an epitope capable of causing the generation of an antibody, or the antibody itself, to effect specific pharmaceutical outcomes (active as well as passive immunisation) from binding to all members of the proteins with a selected conformation. An example of this would be prion proteins in the conformation that leads to the condition vCJD. The abnormal form of the protein could be targeted by a specific antibody or epitope causing the generation of the antibody, preferably human and reduced in size for optimum pharmacological effect.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows the amino acid sequence of the human $P2X_7$ receptor (prior art). Sequences 65 to 81 and 200 to 216 are highlighted and are referred to below.

DETAILED DESCRIPTION OF THE INVENTION

To raise the antibody specifically to non-functional $P2X_7$, the epitope used was the sequence 200 to 216 in Figure 1, containing a Cys at 216.

To raise the antibody to non-discriminatory $P2X_7$, the epitope used was the sequence 65 to 81 in Figure 1, to which was added an N-terminal Cys. This antibody could not detect whether the receptor was non-functional but was designed to detect all receptor so that the proportion of receptor that was functional could be determined by comparing the staining obtained by using the two antibodies separately.

The Cys residues on the epitopes were coupled via a maleimidocaproyl-N-hydroxysuccinimide (MCS) cross linker to diphtheria toxin (DT) carrier with ten peptide epitopes attached to each DT carrier, to maintain conformational stability and provide a larger antigenic structure. These conjugated epitopes were used as the antigens for injection into several animal species (sheep, rabbit and mouse) to raise antibodies specific to the epitopes, in the usual manner.

The procedure for raising antibodies is well documented in the prior art by use of antigen/adjuvant mixtures injected into animals at particular times. Specific examples for raising the antibodies are set out below:

Example 1

Sheep anti-$P2X_7$ antibodies

500 µg of conjugate (approximately 100 µg of $P2X_7$ epitope) was diluted in phosphate-buffered saline (PBS) to 0.8 mL and was emulsified with 1.2 mL of Freund's Complete adjuvant. Sheep were injected at multiple sites both

subcutaneously and intramuscularly with the antigen/adjuvant emulsion. Eight weeks later the sheep were again injected with the same amount of conjugate emulsified with Freund's Incomplete adjuvant at multiple sites. This was repeated 4 weeks later and the animals were bled from the jugular vein. The serum collected was tested for antibody specificity. The sheep were then routinely injected and bled at eight week intervals to provide a pool of serum containing the specific antibodies.

Other sheep were injected with the same dose of conjugated antigen similar to the schedule above but a different adjuvant was used. In these animals, 0.7 mL of the diluted antigen was mixed with 0.1 mL of a Quill A / DEAE Dextran solution (2.5 mg Quill A + 25 mg DEAE Dextran per mL of PBS) and 1.2 mL of ISA 50V Montanide. The emulsion was injected at multiple sites both subcutaneously and intramuscularly. The antibodies produced using this adjuvant produced the same specificities as those produced using Freund's adjuvant.

Example 2

Rabbit anti-$P2X_7$ antibodies

Antibodies were raised in rabbits using the same two adjuvants as with the sheep and the same injection schedules, the only difference being that 300 µg amounts of the conjugate were used for the injection. The antibodies raised had the same specificities as those produced in the sheep and could readily discriminate between the epitopes against which they were raised.

Example 3

Mice anti-$P2X_7$ antibodies

Antibodies were raised in mice against the conjugated epitopes and also against the unconjugated epitope of the non-functional $P2X_7$ epitope (which is able to discriminate receptors that cannot from pores and thus fail to be apoptotic).

In these experiments, the adjuvant used was the QAIGEN Pty Ltd product, ImmunEasy™ which contains the immuno-stimulatory product CpG DNA (trademark of Coley Pharmaceutical Group Inc.)

5 µg of epitope or conjugated epitope was diluted in 70 µL of PBS and 30 µL of ImmunEasy™ adjuvant. Mice were injected at multiple sites subcutaneously and intramuscularly. This regime was repeated two weeks later and again at a further two weeks. Mice were bled eight days after the third injection. Antibodies raised in mice by this method were again able to discriminate between the different $P2X_7$ epitopes and the antibodies against the $P2X_7$ non-functional epitope gave the same results as those raised in sheep and rabbits.

As the above Examples illustrate, antibodies to various epitopes of the $P2X_7$ receptor in different species and using different adjuvants may be raised consistently. In particular, antibodies to an epitope of the $P2X_7$ receptor which identifies the receptor in the non-functional state, in which it cannot form a pore and carry out its apoptotic function under normal physiological conditions, may be raised rountinely.

Example 4

The antibody detecting non-functional $P2X_7$ was tested by binding the antibody to cells expressing $P2X_7$ (human) with known function as revealed through the ability of the $P2X_7$ to take up ethidium or rubidium. These $P2X_7$ protein channels may have been mutated at base pair 1513, such that the channels would not form apoptotic pores. These and similar non-functional $P2X_7$ receptors expressed on malignant B lymphocytes also bound the antibody in flow cytometry and in standard immunohistochemistry while cells expressing normal functional $P2X_7$ (capable of taking up calcium, ethidium and rubidium with large fluxes) were unable to bind the antibody, because the epitope chosen to detect the non-functional receptors was unavailable in functional receptors. The Pro210 adopted a cis conformation in the non-functional receptors and it was specifically this

conformation that was stabilised in the conjugated epitope used to raise the antibody. The Pro210 was in the trans conformation in the receptors that were shown to be functional. This was a result of the binding of ATP (adenosine triphosphate) to the $P2X_7$ receptor. When ATP was bound, the Pro210 on a segment immediately adjacent to the ATP binding site adopted a trans configuration.

This was verified using site directed mutagenesis to change the Pro210 to an Ala that was fixed in the trans configuration and this mutant protein was found to be fully functional and unable to bind the antibody raised to detect the non-functional receptor.

Example 5

Further verification of the specificity of the antibody to detect the non-functional receptor came in experiments that labelled macrophages expressing $P2X_7$. The macrophages bound antibody to the $P2X_7$ receptors using the $P2X_7$ universal antibody but did not bind the antibody to non-functional $P2X_7$ until they had been exposed to cancer cells such as mouse hybridoma cells. Contact between the macrophages and the hybridoma cells induced the expression on the macrophages of non-functional $P2X_7$ that was detected by the antibody to non-functional $P2X_7$ as well as the universal $P2X_7$ antibody.

The macrophages and B-cell lymphocytes extracted from patients with malignant lymphoma were tested and all these cells bound the antibody to universal $P2X_7$ as well as the antibody to the non-functional $P2X_7$ receptors, verifying that $P2X_7$ was non-functional in all the cancer cells detected, with the apoptotic pore formed by functional $P2X_7$ unable to form and thus induce apoptosis in cancer cells.

All such cancer cells from all epithelial cell cancers in humans such as prostate, breast, bowel, skin, stomach, cervix and others as well as malignant lymphoma, chronic lymphocytic leukaemia and brain tumours, as well as the same tumours in

other mammals that were tested, including breast and prostate in dog and skin in cat as well as all mouse hybridoma cells and mouse fibrosarcoma cells, all express the same non-functional $P2X_7$. Sequence similarity between human, rat, cat, dog and mouse at the chosen epitopes is sufficient for positive identification to be made in all the above cases. This shows that the mechanism of cancer in these mammals is identical in that all cancer cells express non-functional $P2X_7$ receptors unable to form apoptotic pores that would normally kill the cell when activated. In this way the cancer cells become immortal, with apoptosis being switched off.

Example 6

As further verification that the cancer cells such as affected B-cell lymphocytes are unable to induce apoptosis through $P2X_7$ function, B cells from leukaemia patients containing non-functional $P2X_7$ receptors were incubated with 5 mM ATP for 2 hours in culture. The results were that all the non-functional receptors were forced by the excess ATP to open and induce apoptosis that killed the affected cells.

Example 7

As further verification that the antibody selectively binds cancer cells, skin from patients with basal cell carcinomas (BCC) were treated with the antibody to the non-functional $P2X_7$ receptors, suspended in an inert cream base and applied to the lesion and surrounding skin (refer Example 10, below). Within 1 week of daily application of the topical antibody, all trace of the BCCs had disappeared with no effect on surrounding skin since normal skin was devoid of the receptors.

DIAGNOSTIC APPLICATIONS

Descriptions are provided here by way of example, using the specific non-functional $P2X_7$ antibody in animals and demonstrating the universal application of the probe and method of the invention to the diagnosis of most cancers in humans and other mammals.

In prostate tissue from humans and mammals, such as cats and dogs, when the antibody of the invention is used for diagnosis, no labelling is obtained in the absence of cancer or pre-cancerous lesions. However, the diagnostic method of the invention reveals first signs of neoplastic change while there is still no accompanying morphological changes detectable by H&E stain.

At this stage, it is necessary to stain for the receptor units first appearing in the nuclei of epithelial cells. These migrate to the cytoplasm in later stages of the disease, acting as a field effect throughout the prostate, so that less tissue need be biopsied to be certain of the existence of a tumour. In later stages of the disease, the staining becomes more confined to the apical epithelium.

Similarly, other epithelial cell cancers, like breast, lung, colon and skin in humans and in other mammals, such as cats and dogs, can be detected with margins as there is no longer a clear field effect in these other tissues.

The same stage development is seen in these other tissues, like breast and cervix, with nuclear stain preceding cytoplasmic stain, while normal tissue is unstained. Affected ducts and lobules in breast tissue are readily detected due to the local field effect within the individual affected duct system in the breast even where normal morphology suggests there is no cancer. Adjacent unaffected ducts appear unstained. Similarly, affected lymph nodes, directly draining tissue containing a tumour, show signs of the tumour through the field effect of affected lymphocytes. Thus, sentinel nodes can be detected without there being any metastatic cellular spread to the node.

Skin cancers, such as basal cell carcinoma, squamous cell carcinoma and dysplastic naevi as well as malignant melanomas show positive staining for non-functional receptors and channel components (monomers) in keratinocyte and melanocyte layers with clear margins beyond which normal skin is unlabelled on both epidermis and deep within the dermis.

All tested mammalian cancer cell lines such as human prostate (PC3) and breast (MCF7) and rodent hybridomas are positive for the non-functional receptors on the cell surface so that apoptosis is inhibited in these cancer cells. The general application of this diagnostic is seen by way of the same label on mouse hybridoma cells showing the ubiquitous nature of the receptor in other animal types besides human. Normal human B-cell lymphocytes show that functional $P2X_7$ receptors are expressed on the cell surface, so enabling apoptosis when necessary, while human B-cell lymphocytes from patients with malignant lymphoma show that non-functional $P2X_7$ receptors are expressed on the cell surface, so curtailing apoptosis.

THERAPEUTIC APPLICATIONS

Targeting this apparently ubiqitous $P2X_7$ non-functional conformer expressed on the cell surface of cancer cells attempting to undergo apoptosis may be used to treat most cancers in humans and other mammals. Examples are set out below:

Example 8

Mouse hybridoma cells were grown on a macrophage base both in the presence and absence of affinity purified antibody to non-functional $P2X_7$. Cell counts revealed that over 4 days while cells coincubated with purified normal IgG grew from 1×10^4 to 7×10^4, coincubation with non-functional $P2X_7$ antibody kept the cell count to only 1.5×10^4.

Example 9

This example shows that antibodies raised against the non-functional epitope of the $P2X_7$ receptor can inhibit tumour formation *in vivo*.

As shown above, antibodies raised in sheep against the non-functional $P2X_7$ epitope identified this non-functional $P2X_7$ apoptotic receptor on the surface of mouse hybridoma cells. Addition of this antibody to hybridoma cell cultures

retarded the growth of the cells. Mouse hybridoma cells when injected into prepared inbreed mouse strains will cause tumour formation.

In this experiment, three groups of 10 Balb-c female mice each received the following treatments:

Group 1: 10 mice each injected intraperitoneally (IP) with 1 x 10^6 hybridoma cells in 0.5 mL of cell culture medium on Day 1. On Days 2 and 3, they received an intraperitoneal injection of 0.5 mL of cell culture medium.

Group 2: 10 mice each injected intraperitoneally (IP) with 1 x 10^6 hybridoma cells in 0.5 mL of cell culture medium containing 1 mg of purified sheep IgG on Day 1. On Days 2 and 3, they were injected with 0.5 mL of cell culture medium containing 1 mg of purified sheep IgG.

Group 3: 10 mice each injected intraperitoneally (IP) with 1 x 10^6 hybridoma cells in 0.5 mL of cell culture medium containing 1 mg of purified sheep anti-$P2X_7$ non-functional epitope IgG on Day 1. On Days 2 and 3, they received a further injection of 0.5 ml of cell culture medium containing 1 mg of purified sheep anti-$P2X_7$ IgG.

Mice from all the groups were killed on Day 11 and examined for the presence of tumour. The tumours were excised and weighed.

The results were as follows:

Groups	Observations	Mean Tumour Weight per mice (± SD) (g)
1: Control 1	9 out of 10 mice had tumours.	3.98 ± 1.1

2: Control 2	10 out of 10 mice had tumours	2.93 ± 0.9
3: Experimental	9 out of 10 mice had tumours	1.13 ± 0.4

An analysis of variance showed a significant difference in tumour weight between the groups (probability $P < 0.01$). The experimental group treated with the anti-$P2X_7$ non-functional antibodies was significantly different ($P < 0.01$) from the two control groups. That is, treatment with antibodies against the $P2X_7$ non-functional epitope significantly reduced the amount of tumour in the experimental animals.

Example 10

Specific affinity purified antibody (to greatly improve specificity) was applied to 3 human basal cell carcinomas ("BCC") either as a liquid held in place for 7 days or suspended in a dimethicone cream base. No trace of the BCC lesions was detectable after treatment, while control skin was entirely unaffected due to the absence of the protein target.

Example 11

Skin lesions of the form of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) (both primary tumours and secondary tumours), including relapsed tumours and dysplastic naevi, were treated in a further trial using purified antibody, IgG either affinity purified or not, mixed in dimethicone cream base or a penetrating cream base. Since there were no non-functional receptors present in the normal skin there were no side effects detected in normal skin of any kind. The cancers of all types all responded to the presence of the antibody by disappearing within a period from thirty six hours to one week with twice daily applications. No relapse has occurred in periods of up to twelve months. The size of the tumours treated ranged from 3mm diameter with no raised border to 5cm diameter and up to 4mm thick. A total of thirty four histologically confirmed tumours have been successfully eliminated within one week treatment periods.

It is believed that application to patients in general would involve production of a human monoclonal antibody (such as herceptin) so that internal cancers could be treated with the same efficacy as is revealed with topical application. All normal functional $P2X_7$ expressed on the cell surfaces of cells such as lymphocytes would need to remain unaffected by the presence of the antibody to avoid side effects. The antibody should therefore only bind to proteins expressed on the cell surface of cells attempting to but unable to initiate apoptosis. Thus all cells targeted would be only those attempting to kill themselves through programmed cell death, including cancer cells. The $P2X_7$ receptors on these cells, particularly cancer cells, would be in a non-functional or ATP-depleted state.

ACTIVE IMMUNISATION

Active immunisation may also be used for therapeutic purposes. In this case the humans or other mammals need to be immunised against a specific epitope or epitopes that are in a conformation that mimics the conformation adopted only by the receptors in their non-functional (ATP-depleted) shape on the cell surface. Conformational flexibility that includes partial exposure of an epitope shape that is present in functional receptors should be avoided. The cis configuration of the epitope Gly200-Cys216 as an example should be fixed before use by appropriate means. As added proof that this concept is sound is the observation that numerous animals including mice, rabbits and sheep used to raise the antibodies have not been immuno-compromised. None of these many animals have ever developed any tumours.

A specific example illustrates this:

Example 12

Protocol: The experiment was conducted on the basis of a mouse tumour model. Forty ten-week old female inbred Balb C mice were used, and divided into two groups of twenty, Group 1 being experimental and Group 2 being the control group.

Day 1: The twenty experimental animals in Group 1 were injected with 0.1 mg of the peptide epitope ($hP2X_7$ sequence 200-216) conjugated to diphtheria toxin via the MCS crosslinker. This contained approximately 0.02 mg of the peptide epitope. The peptide conjugate was emulsified with a QUILL A/DEAE Dextran/Montanide ISA 50V adjuvant mix and injected in a volume of 0.1 mL at multiple subcutaneous and intramuscular sites.

The twenty mice in the control group, Group 2, were injected with 0.1 mL of the adjuvant mix without peptide conjugate at multiple subcutaneous and intramuscular sites.

Day 8: The twenty Group 1 mice were injected with 0.01 mg of the peptide epitope ($hP2X_7$ sequence 200-216) conjugated to diphtheria toxin via the MCS crosslinker (containing approximately 0.002 mg of the peptide epitope). The peptide was contained in a phosphate buffered saline solution and mixed according to the protocol with the commercially available CpG DNA adjuvant ImmunEasy (from Qiagen). A volume of 0.1 mL of peptide conjugate/adjuvant solution was injected at multiple subcutaneous and intramuscular sites in each mouse.

The twenty Group 2 mice were injected with the comparable phosphate buffered saline/ CpG DNA adjuvant mix. This was injected in a volume of 0.1 mL in each mouse at multiple subcutaneous and intramuscular sites.

Day 26: The twenty Group 1 mice were injected with 0.025 mg of the peptide epitope ($hP2X_7$ sequence 200-216) conjugated to diphtheria toxin via the MCS crosslinker (containing approximately 0.005 mg of the peptide epitope). This was contained in a phosphate buffered saline solution and mixed with the Qiagen CpG DNA adjuvant ImmunEasy. Again 0.1 mL of the mix was injected in each mouse at multiple subcutaneous and intramuscular sites. The control group was injected as before on Day 8.

Day 29: All mice received an injection of tumour cells at a single subcutaneous site located at the back of the neck in 0.1 mL of tissue culture media. The tumour cells used were a mouse fibrosarcoma cell line developed by the Walter and Eliza Hall Institute in Melbourne Australia designated cell line WEHI 164.

The cells were injected at two concentrations into both the experimental and control groups of mice. Each group was subdivided into two. Ten mice from each of the experimental and control groups received 160,000 cells per mouse and ten mice from each group received 320,000 cells per mouse.

The cells from this cell line had previously been tested for the presence of the non-functional $P2X_7$ epitope on their cell surface. This was done using an antibody raised in sheep which specifically recognises the non-functional form of the receptor.

Day 38: All mice were killed and blood collected for analysis of antibodies to the non-functional $P2X_7$ epitope. All mice were weighed and the tumours were excised and weighed.

Results

Group	Control 160,000 cells	Experimental 160,000 cells	Control 320,000 cells	Experimental 320,000 cells
n	10	10	10	10
Mean tumour wt (mg)	599	270	1147	750
SD	307	108	633	363
SEM	97	34	200	115

Analysis of variance of the results showed a statistically significant difference between control and treatment groups and between low and high dose groups

(P=0.0003). The lower dose group showed a larger difference due to the lower tumour load having less effect on the ability of the mice immune systems to cope.

ATP ANALOGUE

The efficacy of use of a synthetic agonist to effectively bind to ATP binding sites on the $P2X_7$ pore, to force the pore to enter the functional state, thereby acting to restore receptor function as well as controlling the growth advantage that $P2X_7$ provides cells, is shown in the following experiment in culture. Tumour B-cells collected from a patient with CLL, when mixed with a similar number of like cells from a normal patient were treated with ATP at 2.5 mM for four hours. No tumour cells remained, only normal cells. The use of ATP or the more selective $P2X_7$ agonist benzoyl, benzoyl ATP is not appropriate *in vivo*. Thus, a selective ATP analogue able to selectively bind to $P2X_7$ at much higher affinity than either ATP or BzATP may be designed to reinstate the process of apoptosis in a range of affected tumour cell types.

INDUSTRIAL APPLICABILITY

The invention in all its aspects has application to the fields of human and veterinary medicine and health, with the potential to enable early and accurate diagnosis of diseases and effective treatment, which in many cases is far less invasive or traumatic than those available in the prior art.

CLAIMS

1. A probe for detection of a disease or condition, the probe being adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors.

2. The probe of claim 1, wherein the probe is adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors.

3. The probe of claim 1, wherein the probe is adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors by detecting change in binding of one or more proteins necessary for pore formation in $P2X_7$ receptors.

4. The probe of claim 3, wherein the probe is adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors by detecting one or more parts of the receptor exposed in the absence of bound ATP.

5. The probe of claim 4, wherein the part includes a $P2X_7$ monomer.

6. The probe of any one of claims 1 to 5 which is natural or artificial.

7. The probe of any one of claims 1 to 5, wherein the probe is an antibody chosen from the group consisting of a polyclonal antibody, a monoclonal antibody, a recombinant antibody, a humanised antibody, a human antibody and a fragment thereof.

8. The probe of claim 7, in which the antibody is directed against an epitope of each receptor located in an extracellular domain adjacent to a site for binding ATP.

9. The probe of any one of claims 1 to 5, wherein the receptors are mammalian $P2X_7$ receptors and the probe is adapted to distinguish between functional

receptors having a sequence in which proline at amino acid 210 is in the trans conformation and non-functional receptors having a sequence in which the proline at amino acid 210 is in the cis conformation acting to alter local protein structure.

10. The probe of any one of claims 1 to 5, wherein the receptors are mammalian $P2X_7$ receptors and the probe is adapted to distinguish between functional receptors having a sequence in which proline at amino acid 199 is in the trans conformation and non-functional receptors having a sequence in which the proline at amino acid 199 is in the cis conformation acting to alter local protein structure.

11. The probe of claim 9, wherein the probe is or includes an antibody raised against an epitope sequence of the $P2X_7$ receptor extending from Gly200 to Cys216.

12. The probe of any one of claims 1 to 11, wherein the disease or condition is chosen from the group consisting of: prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, other epithelial cell cancers, malignant lymphoma, other blood cancers, irritable bowel syndrome and infection by a virus or other pathological organism.

13. The probe of claim 12, wherein the virus or organism is HIV or Mycobacterium tuberculosis.

14. The probe of claim 7, wherein the condition is irritable bowel syndrome and the antibody is capable of detecting other regions of the $P2X_7$ receptor unchanged by functional state by detecting an epitope common to both functional and non-functional conformations.

15. The probe of claim 7 or claim 14, wherein the condition is irritable bowel syndrome and the antibody is used in combination with a second antibody capable of detecting total $P2X_7$ expression.

16. A method for detecting a disease or condition, the method including the steps of:

using the probe claimed in any one of claims 1 to 10 to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors,

providing a receptor expression profile, and

comparing the receptor expression profile with that of a normal profile.

17. The method of claim 16, wherein the receptor expression profile is that of non-functional receptors.

18. The method of claim 16, wherein the probe is adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors.

19. The method of claim 16, wherein the probe is adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors by detecting change in binding of one or more proteins necessary for pore formation in $P2X_7$ receptors.

20. The method of claim 16, wherein the probe is adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors by detecting one or more parts of the receptor exposed in the absence of bound ATP.

21. The method of claim 16, wherein the part includes a $P2X_7$ monomer.

22. The method of any one of claims 16 to 21, wherein the receptor expression profile is provided using *in situ* imaging techniques.

23. The method of any one of claims 16 to 21, wherein the receptor expression profile is provided using microscopy, confocal microscopy or fluorescence activated cell sorting.

24. Use of the probe claimed in any one of claims 1 to 15 to detect a disease or condition.

25. An isolated cell or tissue sample complexed with a probe as claimed in any one of claims 1 to 15.

26. A method of diagnosing irritable bowel syndrome, comprising detecting the $P2X_7$ expression profile of cells and/or tissue and comparing the profile with a predetermined expression profile of normal cells and/or tissue.

27. The method of claim 26, wherein the detection of the $P2X_7$ expression profile includes use of one or more antibodies.

28. Use of one or more antibodies to diagnose irritable bowel syndrome.

29. A method of treating irritable bowel syndrome, the method including administering a composition adapted to restore $P2X_7$ receptor function.

30. An antibody for treating or preventing a disease or condition, the antibody being adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors and to bind only to non-functional receptors.

31. The antibody of claim 30, wherein the antibody is adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors by detecting change in relation to binding of adenosine triphosphate (ATP) to the receptors.

32. The antibody of claim 30, wherein the antibody is adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors by detecting change in binding of one or more proteins necessary for pore formation in $P2X_7$ receptors.

33. The antibody of claim 30, wherein the antibody is adapted to distinguish between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors by

detecting one or more parts of the receptor exposed in the absence of bound ATP.

34. The antibody of claim 30, wherein the part includes a $P2X_7$ monomer.

35. The antibody of any one of claims 30 to 34 which is polyclonal, monoclonal, recombinant, humanised or a human antibody or an appropriate fragment thereof.

36. The antibody of any one of claims 30 to 34, wherein the receptors are mammalian $P2X_7$ receptors and the antibody is adapted to distinguish between functional receptors having a sequence in which proline at amino acid 210 is in the trans conformation and non-functional receptors having a sequence in which the proline at amino acid 210 is in the cis conformation.

37. The antibody of claim 36, which is raised against an epitope sequence of the $P2X_7$ receptor extending from Gly200 to Cys216.

38. The antibody of any one of claims 30 to 34, wherein the receptors are mammalian $P2X_7$ receptors and the antibody is adapted to distinguish between functional receptors having a sequence in which proline at amino acid 199 is in the trans conformation and non-functional receptors having a sequence in which the proline at amino acid 199 is in the cis conformation

39. The antibody of any one of claims 30 to 37, wherein the disease or condition is chosen from the group consisting of: prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, other epithelial cell cancers, malignant lymphoma, other blood cancers, irritable bowel syndrome and infection by a virus or other pathological organism.

40. The antibody of claim 39, wherein the virus or organism is HIV or Mycobacterium tuberculosis.

41. An epitope adapted to cause the generation of the antibody of any one of claims 30 to 40.

42. The epitope of claim 41 which is attached to diphtheria toxin via the C-terminal Cys residue by means of the chemical cross-linker maleimidocaproyl-N-hydroxysuccinimide (MCS), so that the conformation adopted by the attached epitope peptide occupies a stable cis proline configuration.

43. Use of the antibody of any one of claims 30 to 40 as a therapeutic vehicle for treatment of a disease or condition in a patient to regulate programmed cell death by targeting aberrant or non-functional $P2X_7$ receptors expressed on the surface of cells, while leaving all cells expressing normal (functional) receptors untouched.

44. Use of the epitope of claim 41 or 42 to cause the generation of the antibody of any one of claims 30 to 40.

45. A pharmaceutical composition for treatment or prevention of a disease or condition in a patient, the composition including a pharmaceutically effective amount of an antibody as claimed in any one of claims 30 to 40, or an epitope to cause the generation of such an amount, capable of regulating programmed cell death of cells having expressed on their surface aberrant or non-functional $P2X_7$ receptors.

46. A preparation for treatment or prevention of a disease or condition in a patient, the preparation including one or more substances adapted to regulate the expression of ATPases or ATPDases that control the supply of ATP to $P2X_7$ receptors in the patient's cells or tissues.

47. A method of treating or preventing a disease or condition in a patient, the method including the step of administering to the patient a preparation including one or more substances adapted to regulate the expression of

ATPases or ATPDases that control the supply of ATP to $P2X_7$ receptors in the cells or tissue of the patient.

48. The preparation of claim 46, wherein the disease or condition is chosen from the group consisting of: prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, other epithelial cell cancers, malignant lymphoma, other blood cancers, irritable bowel syndrome and infection by a virus or other pathological organism.

49. The method of claim 47, wherein the disease or condition is chosen from the group consisting of: prostate, breast, skin, lung, cervix, uterus, stomach, oesophagus, bladder, colon and vaginal cancers, other epithelial cell cancers, malignant lymphoma, other blood cancers, irritable bowel syndrome and infection by a virus or other pathological organism.

50. The antibody of any one of claims 35 to 40, wherein the antibody is a humanised or human antibody or fragment thereof, when used for *in vivo* diagnosis or treatment.

51. The antibody of claim 50, when combined with a radiolabel suitable for detection by use of scanning technology.

52. The antibody of claim 51, when the scanning technology is positron emission tomography.

53. The antibody of claim 50, when combined with a fluorescent label suitable for use in flow cytometry.

54. The antibody of claim 50, when combined with a matrix suitable for colorimetric assay.

55. A test kit for detecting non-functional $P2X_7$ receptors, the kit including the probe of any one of claims 1 to 15, together with a normal $P2X_7$ receptor expression profile.

56. A test kit for detecting non-functional $P2X_7$ receptors, the kit including the antibody of any one of claims 30 to 40, together with a normal $P2X_7$ receptor expression profile.

57. A test kit for detecting non-functional $P2X_7$ receptors, the kit including the antibody of any one of claims 50 to 54, together with a normal $P2X_7$ receptor expression profile.

58. The test kit of claim 56 or 57, when adapted to detect non-functional $P2X_7$ receptors in a blood sample.

59. The preparation of claim 46 or 48, in which the ATPases and ATPDases are chosen from CD39 and CD73.

60. The preparation of any one of claims 46, 48 and 59, wherein the one or more substances is one or more ATP analogues.

61. A method of treating or preventing a disease or condition, the method including use of the antibody claimed in any one of claims 30 to 40.

62. A method of treating or preventing a disease or condition, the method including use of the epitope of claim 41 or 42.

63. A method of treating or preventing a disease or condition, the method including use of the pharmaceutical composition of claim 45.

64. A method of treating or preventing a disease or condition, the method including use of the preparation of claim 46 or 48.

65. The probe of claim 14, wherein the epitope is Val65-Lys81.

66. The antibody of claim 30 substantially as herein described with reference to any one of examples 1 to 3 herein.

67. A method of treating or preventing a disease or condition in a patient substantially as herein described with reference to example 10 or 11 herein.

1 MET-PRO-ALA-CYS-CYS-SER-CYS-SER-ASP-VAL-PHE-GLN-TYR-GLE-THR-ASN-LYS-VAL-
THR-ARG
21 ILE-GLN-SER-MET-ASN-TYR-GLY-THR-ILE-LYS-TRP-PHE-PHE-HIS-VAL-ILE-ILE-PHE-SER-TYR
41 VAL-CYS-PHE-ALA-LEU-VAL-SER-ASP-LYS-LEU-TYR-GLN-ARG-LYS-GLU-PRO-VAL-ILE-SER-SER
61 VAL-HIS-THR-LYS-[redacted]
[redacted]-LYS-LEU-VAL-HIS-SER-VAL-PHE-ASP-THR-ALA-ASP-TYR-THR-PHE-PRO-LEU-GLN-GLY-ASN
101 SER-PHE-PHE-VAL-MET-THR-ASN-PHE-LEU-LYS-THR-GLU-GLY-GLN-GLU-GLN-ARG-LEU-CYS-PRO
121 GLU-TYR-PRO-THR-ARG-ARG-THR-LEU-CYS-SER-SER-ASP-ARG-GLY-CYS-LYS-LYS-GLY-TRP-MET
141 ASP-PRO-GLN-SER-LYS-GLY-ILE-GLN-THR-GLY-ARG-CYS-VAL-VAL-HIS-GLU-GLY-ASN-GLN-LYS
161 THR-CYS-GLU-VAL-SER-ALA-TRP-CYS-PRO-ILE-GLU-ALA-VAL-GLU-GLU-ALA-PRO-ARG-PRO-ALA
181 LEU-LEU-ASN-SER-ALA-GLU-ASN-PHE-THR-VAL-LEU-ILE-LYS-ASN-ASN-ILE-ASP-PHE-PRO-[redacted]
[redacted]-THR-PHE-HIS-LYS
221 THR-GLN-ASN-PRO-GLN-CYS-PRO-ILE-PHE-ARG-LEU-GLY-ASP-ILE-PHE-ARG-GLU-THR-GLY-ASP
241 ASN-PHE-SER-ASP-VAL-ALA-ILE-GLN-GLY-GLY-ILE-MET-GLY-ILE-GLU-ILE-TYR-TRP-ASP-CYS
261 ASN-LEU-ASP-ARG-TRP-PHE-HIS-HIS-CYS-HIS-PRO-LYS-TYR-SER-PHE-ARG-ARG-LEU-ASP-ASP
281 LYS-THR-THR-ASN-VAL-SER-LEU-TYR-PRO-GLY-TYR-ASN-PHE-ARG-TYR-ALA-LYS-TYR-TYR-LYS
301 GLU-ASN-ASN-VAL-GLU-LYS-ARG-THR-LEU-ILE-LYS-VAL-PHE-GLY-ILE-ARG-PHE-ASP-ILE-LEU
321 VAL-PHE-GLY-THR-GLY-GLY-LYS-PHE-ASP-ILE-ILE-GLN-LEU-VAL-VAL-TYR-ILE-GLY-SER-THR
341 LEU-SER-TYR-PHE-GLY-LEU-ALA-ALA-VAL-PHE-ILE-ASP-PHE-LEU-ILE-ASP-THR-TYR-SER-SER
361 ASN-CYS-CYS-ARG-HIS-HIS-ILE-TYR-PRO-TRP-CYS-LYS-CYS-CYS-GLN-PRO-CYS-VAL-VAL-ASN
381 GLU-TYR-TYR-TYR-ARG-LYS-LYS-CYS-GLU-SER-ILE-VAL-GLU-PRO-LYS-PRO-THR-LEU-LYS-TYR
401 VAL-SER-PHE-VAL-ASP-GLU-SER-HIS-ILE-ARG-MET-VAL-ASN-GLN-GLN-LEU-LEU-GLY-ARG-SER
421 LEU-GLN-ASP-VAL-LYS-GLY-GLN-GLU-VAL-PRO-ARG-PRO-ALA-MET-ASP-PHE-THR-ASP-LEU-SER
441 ARG-LEU-PRO-LEU-ALA-LEU-HIS-ASP-THR-PRO-PRO-ILE-PRO-GLY-GLN-PRO-GLU-GLU-ILE-GLN
461 LEU-LEU-ARG-LYS-GLU-ALA-THR-PRO-ARG-SER-ARG-ASP-SER-PRO-VAL-TRP-CYS-GLN-CYS-GLY
481 SER-CYS-LEU-PRO-SER-GLN-LEU-PRO-GLU-SER-HIS-ARG-CYS-LEU-GLU-GLU-LEU-CYS-CYS-ARG
501 LYS-LYS-PRO-GLY-ALA-CYS-ILE-THR-THR-SER-GLU-LEU-PHE-ARG-LYS-LEU-VAL-LEU-SER-ARG
521 HIS-VAL-LEU-GLN-PHE-LEU-LEU-LEU-TYR-GLN-GLU-PRO-LEU-LEU-ALA-LEU-ASP-VAL-ASP-SER
541 THR-ASN-SER-ARG-LEU-ARG-HIS-CYS-ALA-TYR-ARG-CYS-TYR-ALA-THR-TRP-ARG-PHE-GLY-SER
561 GLN-ASP-MET-ALA-ASP-PHE-ALA-ILE-LEU-PRO-SER-CYS-CYS-ARG-TRP-ARG-ILE-ARG-LYS-GLU
581 PHE-PRO-LYS-SER-GLU-GLY-GLN-TYR-SER-GLY-PHE-LYS-SER-PRO-TYR

FIGURE 1

Sequence of human $P2X_7$ receptor.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/01204

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: C07K14/705,16/28; A61K 39/395; A61P 35/00, 1/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CA, MEDLINE, BIOSIS, WPIDS, BIOTECHABS (KEYWORDS: P2X7, ANTIBODY, IMMUNOGLOBIN, CONFORMATION, ATP ANALOGUE, ATPASE, EPITOPE)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Groschel-Stewart U et al, "Localisation of $P2X_5$ and $P2X_7$ receptors by immunohistochemistry in rat stratified squamous epithelia", Cell Tissue Research 1999 vol 296 (3), pages 599-605 See whole document especially page 604 2nd col last para.	1-8, 12-35, 39-64
X	Buell G et al, "Blockade of human $P2X_7$ receptor function with a monoclonal antibody", Blood vol 92 No 10, 1998, pages 3521-3528 See whole document especially page3526 col 2 last para to page 3527 col 2 last para.	1-8, 12-35, 39-64
X	Wiley et al, "A single nucleotide polymorphism is associated with loss of function of the monocyte $P2X_7$ receptor", Blood vol 96 (11 pt 1) Nov 2000 page 17a (Abstract)	1-8, 12-35, 39-64

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 October 2002	22 OCT 2002

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/01204

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Peng L et al, "P2Z purinoceptor, a special receptor for apoptosis induced by ATP in human leukemic lymphocytes", Chinese Medical Journal 1999, 112 (4) pages 356-362 See whole document especially page 361 col 1 para 3.	46-49, 60, 64
P, X	Chessell IP et al, "Dynamics of $P2X_7$ receptor pore dilation: pharmacological and functional consequences", Drug Development Research 2001 vol 53 (2/3) pages 60-65 See whole document especially page 63 col 1 last para to page 64 col 1 2nd para.	1-67
P, X	Wiley JS et al, "Genetic polymorphisms of the human $P2X_7$ receptor and relationship to function", Drug Development Research 2001 vol 53 (2/3) pages 72-76 See whole document especially page 74 col 1 para 2 to page 75 col 1 para 1.	1-67
P, X	WO 02/057306 A1 (INTREAT PTY LTD) 25 July 2002 See whole document	1-67
A	Virgilio F et al, "Purinergic P2X7 receptor: a pivotal role in inflammation and immunomodulation", Drug Development Research 1998 vol 45 (3/4) pages 207-213 See whole document especially page 209 col 1 para 3 to page 210 col 1 para 1.	
A	Ferrari D et al, "ATP-mediated cytotoxicity in microglial cells", Neuropharmacology 1997 vol 36 (9) pages 1295-1301 See whole document.	
A	US 6133434 (G N Buell at al) 17 October 2000 See whole document.	
P, A	Gu B et al, "A Glu-496 to Ala Polymorphism leads to loss of function of the human P2X7 receptor", J. Biol. Chem. 2001 vol 276 (14) pages 11135-11142 See whole document.	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU02/01204

Box I **Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos :

because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos : **1-8, 12-35, 39-64**

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 1-8, 12-35, 39-58, 61-63
It is known that the $P2X_7$ receptors are present in many cell types and that the non-functionality of the receptors is caused by lack of appropriate binding of the ATP agonist to the receptor (see specification

(continue in supplemental box)

3. ☐ Claims Nos :

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II **Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

There are two different groups of inventions as follows:

Group 1. Claims 1-45, 50-58, 61-63 and 66-67 are directed to a probe that distinguishes between functional $P2X_7$ receptors and non-functional $P2X_7$ receptors, method of using the probe in diagnosis and treatment of disease and pharmaceutical composition.

(continue in supplement box)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Supplemental Box
(To be used when the space in any of Boxes I to VIII is not sufficient)

Continuation of Box No: I

page 2 paragraphs 2-3). These claims are mere desiderata and do not contain any technical features to allow for a meaningful search.

Claims 46-49, 60 and 64

It is known that $P2X_7$ receptors are activated by ATP. A preparation that regulates ATPases to control the supply of ATP to $P2X_7$ could include any ATP analogue. No meaningful search can be carried out based on the claims as drafted.

Nonetheless, two searches have been carried out in an attempt to cover the claims.

Continuation of Box No: II

Group 2. Claims 46-49, 60 and 64 are directed to a preparation and method for treatment or prevention of disease that uses one or more substances to regulate the expression of ATPases that control the supply of ATP to $P2X_7$ receptors.

These groups of invention are not so linked to form a single general inventive concept. There are no special technical features that are common to these two groups of claims. Thus the international application does not relate to one invention only or to a single general inventive concept, a priori.

The search result of PCT/AU2002/000061 was considered and therefore this Authority did not invite payment of any additional fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/AU02/01204

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member
WO	2002057306	NONE
US	6133434	NONE

END OF ANNEX